The Angiotensin II Type 2 Receptor Causes Constitutive Growth of Cardiomyocytes and Does Not Antagonize Angiotensin II Type 1 Receptor–Mediated Hypertrophy

Angelo D’Amore, M. Jane Black, Walter G. Thomas

Abstract—Angiotensin II (Ang II) has important actions on the heart via type 1 (AT₁) and type 2 (AT₂) receptors. The link between AT₁ receptor activation and the hypertrophy of cardiomyocytes is accepted, whereas the contribution of the AT₂ receptor, which reportedly antagonizes the AT₁ receptor, is contentious. This ambiguity is primarily based on in vivo approaches, in which the direct effect of the AT₂ receptor and its modulation of the AT₁ receptor (at the level of the cardiomyocyte) are difficult to establish. In this study, we used adenoviruses encoding AT₁ and AT₂ to coexpress these receptors in isolated cardiomyocytes, allowing a direct examination of the consequence of varying AT₁/AT₂ stoichiometry on cardiomyocyte hypertrophy. In myocytes expressing only the AT₁ receptor, Ang II stimulation promoted robust hypertrophy (increased protein:DNA ratio and phenotypic changes) via activation of mitogen-activated protein kinases (MAPKs). Titration of the AT₂ receptor against the AT₁ receptor did not inhibit Ang II–mediated cardiomyocyte hypertrophy. Instead, basal and Ang II–mediated hypertrophy was increased in line with the amplified expression of the AT₂ receptor, indicating a capacity for the AT₂ receptor to enhance basal cardiomyocyte growth. Indeed, expression of the AT₂ receptor alone resulted in hypertrophy; remarkably, this was unaffected by Ang II stimulation or the AT₂ receptor–specific ligands PD123319 and CGP42112. Although previous studies have indicated that the AT₂ receptor can antagonize MAPK activation via the AT₁ receptor, we found no evidence for this in cardiomyocytes. Thus, the AT₂ receptor promotes ligand-independent, constitutive cardiomyocyte hypertrophy and does not directly antagonize the AT₁ receptor in this setting. (Hypertension. 2005;46:1347-1354.)

Key Words: angiotensin II | hypertrophy | myocytes | rats | receptors

Left ventricular hypertrophy (LVH) is a major independent risk factor for premature death.¹ Extensive experimental and clinical evidence supports a role for the vasoactive hormone angiotensin II (Ang II) in the development of hypertension and the associated cardiomyocyte enlargement, which is a hallmark of LVH. Ang II binds with high affinity to type 1 (AT₁) and type 2 (AT₂) Ang II receptors, which are 7-transmembrane spanning, G-protein–coupled receptors.² AT₁ receptors are well characterized and mediate the established actions of Ang II, including vasoconstriction, aldosterone and vasopressin release, renal sodium reabsorption, increased collagen deposition, cellular proliferation, and, importantly, cardiomyocyte hypertrophy. The role of the AT₂ receptor is less clear, but current theories favor a role in opposing the actions of the AT₁ receptor.³⁻⁷ AT₂ receptors are highly expressed in the fetus; however, after birth, the AT₂ receptor expression decreases to low levels.⁵ In normal, adult human and rat hearts, AT₁ and AT₂ receptors are expressed,⁸⁻¹⁰ and they have been shown to be upregulated during cardiovascular pathologies.⁸⁻⁹ The specific signaling pathways activated via the AT₂ receptor remain poorly resolved, although AT₂ receptors reportedly inhibit AT₁ receptor signaling pathways, such as extracellular signal-regulated kinase 1/2 (ERK1/2) mitogen-activated protein kinases (MAPKs), by activating specific tyrosine or serine/threonine phosphatases.⁶¹¹,¹² More recent evidence suggests functional heterodimerization between the AT₁ and AT₂ receptors, in which the AT₂ receptor antagonizes the actions of the AT₁ receptor.¹³

The widely accepted notion that the AT₂ receptor simply counteracts the vasoactive roles of the AT₁ receptor is being increasingly challenged.¹⁴,¹⁵ For example, AT₂ receptors have been shown to decrease⁷¹⁶ or not affect blood pressure and to inhibit,¹⁸⁻²⁰ increase,²¹,²² and not affect²³ collagen deposition. They have been shown to be involved in fetal development²⁴ and cause cellular differentiation.²⁵,²⁶ AT₂ receptors have been reported to inhibit²⁷⁻²⁹ or stimulate vascular growth and angiogenesis, inhibit²⁰⁻²³ and not affect²³ cellular proliferation, and cause apoptosis. Most important, AT₂ receptors have been shown to lead to
either stimulation of,21,22,33 inhibition of,34 or not affect,17,19,32 cardiac hypertrophy. At least part of this inconsistency relates to the experimental approaches used. For example, most of the studies examining the role of the AT<sub>2</sub> receptor in cardiac hypertrophy have been conducted in vivo using wild-type and AT<sub>2</sub> receptor transgenic or knockout animals. Although powerful, such studies can be problematic because of strain differences and complicated by other cellular, circulating, and paracrine factors. Studies aimed at investigating the direct effects of Ang II on cultured cardiomyocytes have also yielded ambiguous results, primarily because of low and variable expression of AT<sub>1</sub> and AT<sub>2</sub> receptors and inconsistent hypertrophy in such models. We have recently shown that Ang II-mediated hypertrophy can be reproducibly demonstrated in cultured cardiomyocytes infected with recombinant AT<sub>2</sub> receptors.35

To directly investigate the effect of activating the AT<sub>2</sub> receptor on cardiomyocyte hypertrophy, we developed an adenovirus expressing the AT<sub>2</sub> receptor, which has allowed us to tightly control the ratio of AT<sub>1</sub>:AT<sub>2</sub> receptor expression in these cells. We report that the AT<sub>2</sub> receptor causes constitutive cardiomyocyte hypertrophy, which is independent of Ang II and in contrast to some prevailing theories, and the AT<sub>2</sub> receptor does not antagonize the hypertrophic actions and signals of the AT<sub>1</sub> receptor.

Materials and Methods

Generation of AdNHA-AT1 and AdNHA-AT2

A plasmid containing the rat AT<sub>1</sub> receptor gene (obtained from Dr T.J. Murphy, Emory University School of Medicine) was subcloned into the mammalian expression vector pRc/CMV at HindIII/XbaI (pRc/CMV-AT2). A hemagglutinin antigen (HA) epitope tag was introduced at the N terminus (pRc/CMV-NHA-AT2) using the ExSite Mutagenesis kit (Stratagene). The recombinant AT<sub>2</sub> receptor adenovirus (AdNHA-AT2) was produced using the method described by He et al.36 The coding region of the NHA-AT2 was introduced at the N terminus (pRc/CMV-NHA-AT2) using the ExSite Mutagenesis kit (Stratagene). The recombinant AT<sub>2</sub> receptor adenovirus (AdNHA-AT2) was produced using the method described by He et al.36 The coding region of the NHA-AT2 was subcloned into the pAdTrack-CMV shuttle vector, obtained from Dr B. Vogelstein36 (Johns Hopkins Oncology Center, Baltimore, Md), using HindIII and XbaI linkers to yield pAdTrackNHA-AT2. pAdTrackNHA-AT2 underwent bacterial homologous recombination with pAdEasy-1, generating recombinant adenoviruses (AdNHA-AT2). AdNHA-AT2 and AdNHA-AT1 viral stock titers used were 0.37×10<sup>10</sup> plaque forming units (PFU)/μL and 0.22×10<sup>10</sup> PFU/μL, respectively (the AT<sub>1</sub> receptor adenovirus [AdNHA-AT1] has been reported previously).35

Cell Culture

Cardiomyocytes isolated from 1-day-old Sprague-Dawley rat ventricles were plated at high density in 12-well plates at 0.475×10<sup>6</sup> cells per well (1250 cells/mm<sup>2</sup> for radio-labeled binding, Western blots, protein, and DNA assays) or at low density at 0.125×10<sup>6</sup> cells per well (330 cells/mm<sup>2</sup>; for phalloidin staining) as described previously.37 Cardiomyocytes plated at high density were grown in modified Eagle’s medium with 10% bovine calf serum for 24 hours, then in defined, serum-free media with KCl (50 mmol/L) to arrest spontaneous beating.37 Animals, supplied by the Precinct Animal Centre (Alfred Medical Research and Education Precinct, Prahran, Melbourne, Australia), were handled in accordance with the Australian code of practice for the care and use of animals for scientific purposes.

Adenoviral Infection of Cardiomyocytes

After 24-hour incubation in defined serum-free media, cardiomyocytes were infected with 3 levels of AdNHA-AT1, termed L<sub>_</sub> (low; multiplicity of infection (MOI)=8; 4×10<sup>6</sup> PFU), M<sub>_</sub> (medium; MOI=23; 11×10<sup>6</sup> PFU), and H<sub>_</sub> (high; MOI=70; 33×10<sup>6</sup> PFU), or with 3 amounts of AdNHA-AT2, L<sub>_</sub> (MOI=31; 15×10<sup>6</sup> PFU), M<sub>_</sub> (MOI=93; 44×10<sup>6</sup> PFU), and H<sub>_</sub> (MOI=280; 133×10<sup>6</sup> PFU). M_AdNHA-AT1 and M_AdNHA-AT2 show similar amounts of green fluorescent protein (GFP) fluorescence (data not shown), indicating similar amounts of viral infectivity.

Characterization of AdNHA-AT2

The AT<sub>1</sub> and AT<sub>2</sub> receptors were detected, quantified, and characterized in AdNHA-AT1–infected and AdNHA-AT2–infected cardiomyocytes 48 hours after infection via equilibrium competition binding assays.35 A [125I]-Ang II tracer was displaced with Ang II, the AT<sub>2</sub> receptor antagonist candesartan (CV-11974; Astra H ässle), the AT<sub>1</sub> receptor antagonist PD123319 (Sigma), and the AT<sub>2</sub> receptor peptide agonist CGP42112A (Bachem). Receptor concentrations were calculated according to Swillens.40 AT<sub>1</sub> and AT<sub>2</sub> receptors were immunoprecipitated from cell extracts of adenoviral-infected cardiomyocytes. The immunoprecipitates were Western blotted using monoclonal anti-HA antibodies (Roche Diagnostics; 1:1000) and chemiluminescence (Pierce).

Cardiomyocyte Hypertrophy

Forty-eight hours after adenoviral infection, cardiomyocytes were stimulated with Ang II (0.1 μmol/L), candesartan (1 μmol/L), PD123319 (1 μmol/L), CGP42112A (0.1 μmol/L), or the MAPK inhibitor PD98059 (Sigma; 20 μmol/L). Blockers were administered 30 minutes before Ang II stimulation, when in combination. Seventy-two hours after stimulation, cardiomyocytes were harvested, and hypertrophy, defined as increased protein:DNA ratio, was determined as described previously.35

Phalloidin Staining

Low-density cardiomyocytes were stained with tetrathymylrhodamine B isothiocyanate (TRITC)–labeled phalloidin (Sigma) as described previously.35,37 This allows a visualization of sarcomeric reorganization, a feature of cardiomyocyte hypertrophy, in these cells.

ERK1/2 Activity

ERK1/2 activation and expression were examined using Western blots probed with monoclonal phospho-p44/42 antibody (Cell Signaling Technology) and anti-ERK1 antibody (Santa Cruz Biotechnology) as described previously.35,37

Statistical Analysis

All data are presented as mean±SEM. For comparisons between 2 groups, a Student t test was used. For all multigroup comparisons, data were analyzed using a 1-way ANOVA followed by a Tukey’s post hoc test. Significance was accepted at P<0.05. Grouped data are from 4 separate experiments, each of which involved triplicate wells assayed in duplicate.

Results

Characterization of AdNHA-AT2

Binding of [125I]-Ang II to AT<sub>1</sub> receptors expressed on cardiomyocytes using the adenovirus was displaced with high affinity by Ang II and the AT<sub>1</sub> receptor specific ligand candesartan but not the AT<sub>2</sub> receptor specific ligands PD123319 and CGP42112A (Figure 1A). [125I]-Ang II binding to AT<sub>2</sub> receptors expressed on cardiomyocytes was displaced with Ang II, PD123319, and CGP42112A but not candesartan, confirming AT<sub>2</sub> receptor adenoviral expression (Figure 1B).

AT<sub>2</sub> receptor expression was titrated with different amounts of AdNHA-AT2, designated low (L<sub>_</sub>), medium

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and high (H; Figure 1C). Infection with M_AdNHA-AT2 resulted in similar to that of M_AdNHA-AT1–infected cells, which produced 500-fmol receptor/mg protein.35 Figure 1D shows expression of the AT1 receptor compared with the titrated AT2 receptor in cardiomyocytes. Receptors were immunoprecipitated and Western blotted for the N-terminal HA epitope. AdNHA-AT2–infected cardiomyocytes showed increased AT2 receptor expression with increased viral titer (Figure 1D); the level of receptor protein generated by M_AdNHA-AT2 was similar to that produced by M_AdNHA-AT1, confirming the radioligand binding data.

Characterization of Uninfected Cardiomyocytes

In uninfected neonatal rat cardiomyocytes, radioligand binding of [125I]-Ang II showed very low to undetectable levels of endogenous AT1 and AT2 receptors (data not shown). These myocytes failed to hypertrophy after Ang II stimulation (Figure 2), as measured by protein content normalized to cellular DNA. We previously demonstrated that these cells are capable of ∼30% hypertrophy by activation of endogenous α1-adrenergic receptors and that the lack of Ang II–induced hypertrophy is attributable to low AT1 receptor expression in these highly purified cultures.35 Low levels of endogenous Ang II receptors in neonatal cardiomyocytes and the absence of Ang II–mediated hypertrophy makes this model ideal for introducing AT1 and AT2 receptors and examining their interactions in a controlled manner.

Hypertrophic Effects of AT1 Receptors

As observed previously,35 increasing the expression of AT1 receptors on cardiomyocytes promoted robust Ang II–stimulated hypertrophy (Figure 3A). However, increasing the expression of the AT1 receptor on cardiomyocytes does not increase basal (in the absence of Ang II) cardiomyocyte hypertrophy. The Ang II–stimulated hypertrophy observed in M_AdNHA-AT1–infected cardiomyocytes (125 ± 3% where uninfected, unstimulated control is 100%) was reversed by candesartan treatment but unaffected by the AT2 receptor ligands (Figure 3B). The phenotypic changes that occur during Ang II–induced hypertrophy of M_AdNHA-AT1–infected cardiomyocytes can be clearly seen by examination of GFP fluorescence (resulting from virus infection) and phalloidin staining (Figure 3C).
Hypertrophic Effects of AT2 Receptors

In contrast to the AT1 receptor, increasing the expression of the AT2 receptor on cardiomyocytes led to a concomitant increase in basal hypertrophy (Figure 4A), which was unaffected by treatment with any of the Ang II receptor ligands (Ang II, PD123319, CGP42112A, or candesartan; Figure 4B). The MAPK inhibitor PD98059 was unable to inhibit this constitutive growth (Figure 4B), although it abrogates Ang II–mediated growth via the AT1 receptor. H_AdNHA-AT2–infected cardiomyocytes show a hypertrophic phenotype, in the absence of Ang II stimulation, when examining GFP labeling and phalloidin staining (Figure 4C).

To confirm that the AT2 constitutive growth did not result from a nonspecific adenoviral effect, we infected cells with a control backbone adenovirus (AdGO; MOI=17; 8×10⁶ PFU, which matched the level of GFP fluorescence of H_AdNHA-AT2) and observed no change in basal hypertrophy compared with the uninfected control (data not shown).

Hypertrophic Effects of Coexpressing the AT1 and AT2 Receptors

We next investigated whether coexpression of the AT2 receptor could modify the hypertrophic response of AT1 receptor activation. As shown in Figure 5, increasing AT2 receptor expression did not inhibit Ang II–mediated hypertrophy through the AT1 receptor. AT2 receptor–mediated enhanced basal hypertrophy was maintained and was additive to that of the AT1 receptor, indicating that these receptors are impinging on separate pathways. The AT1 receptor component was inhibited by candesartan. Treatment with the AT2 receptor antagonist PD123319 did not reverse the Ang II hypertrophic response, and stimulation of the AT2 receptors with the agonist CGP42112A before Ang II stimulation of the AT1 receptors also did not inhibit hypertrophy (Figure 5B).

Figure 2. Uninfected neonatal cardiomyocytes are unresponsive to Ang II or AT1/AT2 agonists/antagonists. Stimulation of uninfected cardiomyocytes with Ang II did not promote hypertrophy and addition of candesartan, PD123319, or CGP42112 had no effect. Note that hypertrophy is represented as percentage differences from the control group.

Figure 3. Increasing amounts of AT1 receptors results in an increasing hypertrophic response to Ang II. A, Ang II–mediated hypertrophy of cardiomyocytes infected with increasing amounts of AdNHA-AT1 receptors (low [L], medium [M], and high [H]). B, Effect on Ang II–induced hypertrophy (black bar) by candesartan (checkered bar), PD123319 (crosshatched), or CGP42112 (vertical stripes) treatments. C, Phenotypic changes of cardiomyocytes visualized via GFP expression after M_AdNHA-AT1 infection (left panels) and via TRITC-labeled phalloidin staining (right panels). Bar=100 μm. *P<0.01 compared with the unstimulated counterpart; **P<0.001 compared with the unstimulated counterpart; †P<0.01 compared with uninfected cardiomyocytes with Ang II stimulation; ††P<0.001 compared with uninfected cardiomyocytes with Ang II stimulation; ‡P<0.005 compared with Ang II stimulated.
Effect of the AT1 and AT2 Receptors on MAPK Activity

Previous studies have indicated that the AT2 receptor may antagonize the capacity of AT1 receptors to activate ERK1/2 MAPK.6 AT1 receptor–mediated ERK1/2 activation after Ang II stimulation was unaffected by coexpression of equal or greater amounts of AT2 receptor (Figure 6).

Effect of the AT1 and AT2 Receptors on MAPK Activity

Previous studies have indicated that the AT1 receptor may antagonize the capacity of AT1 receptors to activate ERK1/2 MAPK.6 AT1 receptor–mediated constitutive hypertrophy was unaffected by cotreatment with AT1 and AT2 receptor ligands or an inhibitor of ERK1/2 signaling. H_AdNHA-AT2 infected cardiomyocytes (GFP-labeled) show a hypertrophic phenotype in the absence of Ang II stimulation (right panel; similar to that seen in Figure 3C). Similarly, TRITC-labeled phalloidin staining (middle panel) shows sarcomeric reorganization not observed in the control uninfected, unstimulated cardiomyocytes (left panel). Bar=100μm. †P<0.01 compared with uninfected, unstimulated control; †P<0.05 compared with uninfected cardiomyocytes with Ang II stimulation; ††P<0.01 compared with uninfected cardiomyocytes with Ang II stimulation.

Discussion

The contribution (either positive or negative) of the AT2 receptor to cardiac hypertrophy is equivocal.5,16,21,22,33,34,41 In the present study, we used cultured neonatal cardiomyocytes (which express low to undetectable levels of Ang II receptors) and recombinant adenoviruses, encoding the AT1 and AT2 receptors, to examine the involvement of the AT2 receptor in cardiomyocyte hypertrophy. The main advantage of our approach is that the degree and stoichiometry of AT1 and AT2 receptor expression can be varied, and it is possible to directly examine the hypertrophy of cardiomyocytes in isolation. Thus, we were able to express different ratios and amounts of AT1 and AT2 receptors and then determine the effect on cardiomyocyte hypertrophy in response to Ang II stimulation and in the presence and absence of AT1 and AT2 selective ligands. We show that increasing the AT2 receptor expression in cardiomyocytes leads to a ligand-independent...
increase in basal hypertrophy. Importantly, we also clearly document that Ang II–stimulated AT1 receptor–mediated hypertrophy of isolated cardiomyocytes is unaffected by coexpression of the AT2 receptor. Thus, in cardiomyocytes, the AT2 receptor causes constitutive growth and does not oppose the actions of the AT1 receptor.

A variety of elegant in vivo experimental approaches (including knockout, transgenic, and lentiviral AT2 receptor expression) have attempted to delineate the role of the AT2 receptor in cardiac hypertrophy. In some studies, abolition or cardiac-specific overexpression of the AT2 receptor failed to implicate this receptor in the modulation of LVH after Ang II infusion or aortic banding. In contrast, in another AT2 receptor knockout model (albeit in a different strain), LVH was prevented after either Ang II infusion or aortic constriction compared with wild-type counterparts. This indicates the apparent importance of the AT2 receptor in the development of LVH. Moreover, Yan et al reported that ventricular-specific AT2 receptor overexpression (using the myosin light chain promoter) leads to an increase in LVH in the absence of any external stimulus. Finally, delivery of AT2 receptors to a subpopulation of cardiomyocytes and presumably other cells (because a cardiac-specific promoter was not used) using a bolus injection of cardiomyocytes expressing only AT1 receptors showed a robust increase in MAPK activity. Coexpression of AT2 receptors in cells not expressing or expressing adenovirus-directed AT1 receptors had no effect on basal or Ang II–stimulated ERK1/2 activation.

Figure 6. AT1–mediated ERK1/2 activity is not altered by AT2 receptor coexpression. ERK1/2 activation and expression were examined using Western blots probed with monoclonal phospho-p44/42 antibody and anti-ERK1 antibody. Ang II stimulation of cardiomyocytes expressing only AT1 receptors showed a robust increase in MAPK activity. Coexpression of AT2 receptors in cells not expressing or expressing adenovirus-directed AT1 receptors had no effect on basal or Ang II–stimulated ERK1/2 activation.

CGP42112) could block or facilitate this effect, indicating that this receptor is constitutively active and that the presence of the AT2 receptor alone is sufficient to drive this process. Although we cannot completely rule out that our results are a nuance of receptor overexpression, such an idea (ie, constitutive activity of the AT2 receptor) has precedence. For example, the AT2 receptor displays ligand pharmacology consistent with it residing in a constant, active conformation. This receptor induces apoptosis in the absence of Ang II stimulation, and the AT2 receptor antagonist PD123319 does not modulate this effect. In addition, Jin et al showed that overexpression of the AT2 receptor downregulates AT1 receptor expression in vascular smooth muscle cells, with and without Ang II stimulation. In contrast to the AT1 receptor, in which signal transduction pathways are well documented, the extent and type of intracellular signals that emanate from the AT2 receptor have been difficult to define. Thus, the molecular mechanisms and signals that generate constitutive activity in the AT2 receptor remain elusive. Recently, it was suggested that in Chinese hamster ovary cells, homo-oligomerization of AT1 receptors leads to constitutive cell signaling. Given this emerging body of evidence, it would seem appropriate to consider reinterpreting some existing data based on consideration of a constitutive rather than ligand-activated AT2 receptor.

Activation of ERK1/2 MAPKs has been strongly associated with hypertrophy of cardiomyocytes, and there is evidence that the AT2 receptor impinges on ERK1/2 activation. Although AT2 receptors have been shown to induce MAPK activity in neuronal NG108–15 cells, most studies have pointed to an inhibitory action of the AT2 receptor on ERK1/2 MAPK and growth, primarily via activation of the SH2 domain-containing tyrosine phosphatase (SHP-1), MAPK phosphatase-1, and serine/threonine phosphatase 2A pathways. Interestingly, Feng et al could provide no evidence to support a constitutive activation of SHP-1 by the AT2 receptor; instead, SHP-1 associated with the “inactive” AT2 receptor and was released on Ang II stimulation. In cardiac fibroblasts, the AT2 receptor inhibited protein tyrosine phosphatases but had no effect on MAPK ERK1/2 activity. Our results show that AT2 receptors alone do not alter ERK1/2 activity, and ERK inhibition did not alter AT2 receptor constitutive growth. Thus, the mechanism of the constitutive cardiomyocyte growth remains to be determined, but it is clearly not via the MAPK pathway. Moreover, the AT2 receptor did not inhibit AT1 receptor–induced ERK1/2 activation, supporting our observation that AT2 coexpression did not affect AT1–mediated hypertrophy. A potential prohypertrophic pathway of the AT2 receptor has been suggested to involve this receptor binding to the promyelocytic zinc finger protein, which is then translocated to the nucleus and activates the p85α phosphatidylinositol 3-kinase gene leading to protein synthesis.

The major outcome of our study is that we can provide no evidence to support the widely stated view that the AT2 receptor opposes the actions of the AT1 receptor. AT1 receptor–mediated cardiomyocyte hypertrophy was unaffected by coexpressing increasing levels of the AT2 receptor. This was an unexpected finding because there is significant
literature suggesting that the AT$_2$ receptor opposes the growth actions of the AT$_1$ receptor, such as antiproliferation in vascular smooth muscle cells, endothelial cells, fibroblasts, and neuronal cells. Indeed, a recent report (that our data call into question) proposed that the AT$_2$ receptor can physically associate as a heterodimer with the AT$_1$ receptor and directly antagonize its actions. Although overexpression of the AT$_2$ receptor using lentivirus injection into the heart was also reported to inhibit/delay Ang II/AT$_1$–dependent cardiac hypertrophy, this likely reflects nonmyocyte actions because expression of the AT$_2$ receptor was not driven by a cardiomyocyte-specific promoter. However, our results are in agreement with transgenic studies in which Ang II–mediated cardiac hypertrophy, via the AT$_1$ receptor, was unaffected by cardiomyocyte-specific overexpression of the AT$_1$ receptor.

In summary, the ambiguous role of the AT$_2$ receptor in cardiomyocyte hypertrophy relates to conflicting interpretations of data from a variety of in vivo and in vitro studies. In this study, we examined the direct role of the AT$_1$ receptor in cardiomyocyte hypertrophy independently of in vivo complications. Using an AT$_2$ receptor expressing adenovirus to infect cardiomyocytes, we report that the AT$_2$ receptor causes constitutive cardiomyocyte hypertrophy via an ERK1/2 MAPK–independent pathway. Finally, our data refute the idea that the AT$_2$ receptor antagonizes the AT$_1$ receptor in the setting of cardiomyocyte hypertrophy or ERK1/2 MAPK activation.

**Perspectives**

The enigmatic nature of the AT$_1$ receptor has complicated a detailed appreciation of its contribution to cardiovascular regulation and, specifically, cardiac hypertrophy. Our data call into question the strongly held view that the AT$_2$ receptor opposes the actions of the AT$_1$ receptor, either by inhibiting AT$_1$–mediated ERK1/2 activation or via direct antagonism mediated by heterodimerization between the 2 receptors. Instead, we favor a model in which both receptors independently impinge on cardiomyocyte growth. The next major challenge in this area will be to unambiguously establish the type and extent of intracellular signals emanating from the AT$_2$ receptor, particularly those that underlie its capacity to promote constitutive cardiomyocyte growth.

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